

SEQUENCE PROTOCOL

<110> Degussa AG

5 <120> Process for the fermentative preparation of
L-amino acids using strains of the
Enterobacteriaceae family.

10 <130> 000425 BT

<140>

<141>

<160> 19

15 <170> PatentIn Ver. 2.1

<210> 1

<211> 1622

20 <212> DNA

<213> Escherichia coli

<220>

<221> CDS

25 <222> (1)..(1620)

<223> pckA

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1 5 10 15

35 atc agt gac gta cat gat atc gtt tac aac cca agc tac gac ctg ctg 96
Ile Ser Asp Val His Asp Ile Val Tyr Asn Pro Ser Tyr Asp Leu Leu
20 25 30

40 tat cag gaa gag ctc gat ccg agc ctg aca ggt tat gag cgc ggg gtg 144
Tyr Gln Glu Glu Leu Asp Pro Ser Leu Thr Gly Tyr Glu Arg Gly Val
35 40 45

45 tta act aat ctg ggt gcc gtt gcc gtc gat acc ggg atc ttc acc ggt 192
Leu Thr Asn Leu Gly Ala Val Ala Val Asp Thr Gly Ile Phe Thr Gly
50 55 60

50 cgt tca cca aaa gat aag tat atc gtc cgt gac gat acc act cgc gat 240
Arg Ser Pro Lys Asp Lys Tyr Ile Val Arg Asp Asp Thr Thr Arg Asp
65 70 75 80

55 act ttc tgg tgg gca gac aaa ggc aaa ggt aag aac gac aac aaa cct 288
Thr Phe Trp Trp Ala Asp Lys Gly Lys Gly Lys Asn Asp Asn Lys Pro
85 90 95

60 ctc tct ccg gaa acc tgg cag cat ctg aaa ggc ctg gtg acc agg cag 336
Leu Ser Pro Glu Thr Trp Gln His Leu Lys Gly Leu Val Thr Arg Gln
100 105 110

65 ctt tcc ggc aaa cgt ctg ttc gtt gtc gac gct ttc tgt ggt gcg aac 384
Leu Ser Gly Lys Arg Leu Phe Val Val Asp Ala Phe Cys Gly Ala Asn
115 120 125

60

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ccg gat act cgt ctt tcc gtc cgt ttc atc acc gaa gtg gcc tgg cag 432
Pro Asp Thr Arg Leu Ser Val Arg Phe Ile Thr Glu Val Ala Trp Gln
130 135 140

5 gcg cat ttt gtc aaa aac atg ttt att cgc ccg agc gat gaa gaa ctg 480
Ala His Phe Val Lys Asn Met Phe Ile Arg Pro Ser Asp Glu Glu Leu
145 150 155 160

10 gca ggt ttc aaa cca gac ttt atc gtt atg aac ggc gcg aag tgc act 528
Ala Gly Phe Lys Pro Asp Phe Ile Val Met Asn Gly Ala Lys Cys Thr
165 170 175

15 aac ccg cag tgg aaa gaa cag ggt ctc aac tcc gaa aac ttc gtg gcg 576
Asn Pro Gln Trp Lys Glu Gln Gly Leu Asn Ser Glu Asn Phe Val Ala
180 185 190

20 ttt aac ctg acc gag cgc atg cag ctg att ggc ggc acc tgg tac ggc 624
Phe Asn Leu Thr Glu Arg Met Gln Leu Ile Gly Gly Thr Trp Tyr Gly
195 200 205

ggc gaa atg aag aaa ggg atg ttc tcg atg atg aac tac ctg ctg ccg 672
Gly Glu Met Lys Lys Gly Met Phe Ser Met Met Asn Tyr Leu Leu Pro
210 215 220

25 ctg aaa ggt atc gct tct atg cac tgc tcc gcc aac gtt ggt gag aaa 720
Leu Lys Gly Ile Ala Ser Met His Cys Ser Ala Asn Val Gly Glu Lys
225 230 235 240

30 ggc gat gtt gcg gtg ttc ttc ggc ctt tcc ggc acc ggt aaa acc acc 768
Gly Asp Val Ala Val Phe Phe Gly Leu Ser Gly Thr Gly Lys Thr Thr
245 250 255

35 ctt tcc acc gac ccg aaa cgt cgc ctg att ggc gat gac gaa cac ggc 816
Leu Ser Thr Asp Pro Lys Arg Arg Leu Ile Gly Asp Asp Glu His Gly
260 265 270

40 tgg gac gat gac ggc gtg ttt aac ttc gaa ggc ggc tgc tac gca aaa 864
Trp Asp Asp Asp Gly Val Phe Asn Phe Glu Gly Gly Cys Tyr Ala Lys
275 280 285

act atc aag ctg tcg aaa gaa gcg gaa cct gaa atc tac aac gct atc 912
Thr Ile Lys Leu Ser Lys Glu Ala Glu Pro Glu Ile Tyr Asn Ala Ile
290 295 300

45 cgt cgt gat gcg ttg ctg gaa aac gtc acc gtg cgt gaa gat ggc act 960
Arg Arg Asp Ala Leu Leu Glu Asn Val Thr Val Arg Glu Asp Gly Thr
305 310 315 320

50 atc gac ttt gat gat ggt tca aaa acc gag aac acc cgc gtt tct tat 1008
Ile Asp Phe Asp Asp Gly Ser Lys Thr Glu Asn Thr Arg Val Ser Tyr
325 330 335

55 ccg atc tat cac atc gat aac att gtt aag ccg gtt tcc aaa gcg ggc 1056
Pro Ile Tyr His Ile Asp Asn Ile Val Lys Pro Val Ser Lys Ala Gly
340 345 350

cac gcg act aag gtt atc ttc ctg act gct gat gct ttc ggc gtg ttg 1104
His Ala Thr Lys Val Ile Phe Leu Thr Ala Asp Ala Phe Gly Val Leu
355 360 365

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 370 375 380

5 tct ggc ttc acc gcc aaa ctg gcc ggt act gag cgt ggc atc acc gaa 1200
 Ser Gly Phe Thr Ala Lys Leu Ala Gly Thr Glu Arg Gly Ile Thr Glu
 385 390 395 400

10 ccg acg cca acc ttc tcc gct tgc ttc ggc gcg gca ttc ctg tcg ctg 1248
 Pro Thr Pro Thr Phe Ser Ala Cys Phe Gly Ala Ala Phe Leu Ser Leu
 405 410 415

15 cac ccg act cag tac gca gaa gtg ctg gtg aaa cgt atg cag gcg gcg 1296
 His Pro Thr Gln Tyr Ala Glu Val Leu Val Lys Arg Met Gln Ala Ala
 420 425 430

20 ggc gcg cag gct tat ctg gtt aac act ggc tgg aac ggc act ggc aaa 1344
 Gly Ala Gln Ala Tyr Leu Val Asn Thr Gly Trp Asn Gly Thr Gly Lys
 435 440 445

cgt atc tcg att aaa gat acc cgc gcc att atc gac gcc atc ctc aac 1392
 Arg Ile Ser Ile Lys Asp Thr Arg Ala Ile Ile Asp Ala Ile Leu Asn
 450 455 460

25 ggt tcg ctg gat aat gca gaa acc ttc act ctg ccg atg ttt aac ctg 1440
 Gly Ser Leu Asp Asn Ala Glu Thr Phe Thr Leu Pro Met Phe Asn Leu
 465 470 475 480

30 gcg atc cca acc gaa ctg ccg gcc gta gac acg aag att ctc gat ccg 1488
 Ala Ile Pro Thr Glu Leu Pro Gly Val Asp Thr Lys Ile Leu Asp Pro
 485 490 495

35 cgt aac acc tac gct tct ccg gaa cag tgg cag gaa aaa gcc gaa acc 1536
 Arg Asn Thr Tyr Ala Ser Pro Glu Gln Trp Gln Glu Lys Ala Glu Thr
 500 505 510

ctg gcg aaa ctg ttt atc gac aac ttc gat aaa tac acc gac acc cct 1584
 Leu Ala Lys Leu Phe Ile Asp Asn Phe Asp Lys Tyr Thr Asp Thr Pro
 515 520 525

40 gcg ggt gcc gcg ctg gta gcg gct ggt ccg aaa ctg taa 1623
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 530 535 540

45 <210> 2
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 <213> Escherichia coli

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Tyr Gln Glu Glu Leu Asp Pro Ser Leu Thr Gly Tyr Glu Arg Gly Val
 35 40 45

60

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Leu Thr Asn Leu Gly Ala Val Ala Val Asp Thr Gly Ile Phe Thr Gly
 50 55 60
 5 Arg Ser Pro Lys Asp Lys Tyr Ile Val Arg Asp Asp Thr Thr Arg Asp
 65 70 75 80
 Thr Phe Trp Trp Ala Asp Lys Gly Lys Gly Lys Asn Asp Asn Lys Pro
 85 90 95
 10 Leu Ser Pro Glu Thr Trp Gln His Leu Lys Gly Leu Val Thr Arg Gln
 100 105 110
 Leu Ser Gly Lys Arg Leu Phe Val Val Asp Ala Phe Cys Gly Ala Asn
 115 120 125
 15 Pro Asp Thr Arg Leu Ser Val Arg Phe Ile Thr Glu Val Ala Trp Gln
 130 135 140
 20 Ala His Phe Val Lys Asn Met Phe Ile Arg Pro Ser Asp Glu Glu Leu
 145 150 155 160
 Ala Gly Phe Lys Pro Asp Phe Ile Val Met Asn Gly Ala Lys Cys Thr
 165 170 175
 25 Asn Pro Gln Trp Lys Glu Gln Gly Leu Asn Ser Glu Asn Phe Val Ala
 180 185 190
 Phe Asn Leu Thr Glu Arg Met Gln Leu Ile Gly Gly Thr Trp Tyr Gly
 195 200 205
 30 Gly Glu Met Lys Lys Gly Met Phe Ser Met Met Asn Tyr Leu Leu Pro
 210 215 220
 35 Leu Lys Gly Ile Ala Ser Met His Cys Ser Ala Asn Val Gly Glu Lys
 225 230 235 240
 Gly Asp Val Ala Val Phe Phe Gly Leu Ser Gly Thr Gly Lys Thr Thr
 245 250 255
 40 Leu Ser Thr Asp Pro Lys Arg Arg Leu Ile Gly Asp Asp Glu His Gly
 260 265 270
 Trp Asp Asp Asp Gly Val Phe Asn Phe Glu Gly Gly Cys Tyr Ala Lys
 275 280 285
 45 Thr Ile Lys Leu Ser Lys Glu Ala Glu Pro Glu Ile Tyr Asn Ala Ile
 290 295 300
 50 Arg Arg Asp Ala Leu Leu Glu Asn Val Thr Val Arg Glu Asp Gly Thr
 305 310 315 320
 Ile Asp Phe Asp Asp Gly Ser Lys Thr Glu Asn Thr Arg Val Ser Tyr
 325 330 335
 55 Pro Ile Tyr His Ile Asp Asn Ile Val Lys Pro Val Ser Lys Ala Gly
 340 345 350
 His Ala Thr Lys Val Ile Phe Leu Thr Ala Asp Ala Phe Gly Val Leu
 355 360 365
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Pro Pro Val Ser Arg Leu Thr Ala Asp Gln Thr Gln Tyr His Phe Leu
370 375 380

5 Ser Gly Phe Thr Ala Lys Leu Ala Gly Thr Glu Arg Gly Ile Thr Glu
385 390 395 400

Pro Thr Pro Thr Phe Ser Ala Cys Phe Gly Ala Ala Phe Leu Ser Leu
405 410 415

10 His Pro Thr Gln Tyr Ala Glu Val Leu Val Lys Arg Met Gln Ala Ala
420 425 430

Gly Ala Gln Ala Tyr Leu Val Asn Thr Gly Trp Asn Gly Thr Gly Lys
435 440 445

15 Arg Ile Ser Ile Lys Asp Thr Arg Ala Ile Ile Asp Ala Ile Leu Asn
450 455 460

20 Gly Ser Leu Asp Asn Ala Glu Thr Phe Thr Leu Pro Met Phe Asn Leu
465 470 475 480

Ala Ile Pro Thr Glu Leu Pro Gly Val Asp Thr Lys Ile Leu Asp Pro
485 490 495

25 Arg Asn Thr Tyr Ala Ser Pro Glu Gln Trp Gln Glu Lys Ala Glu Thr
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530 535 540

35 <210> 3
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40 <220>
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<223> Mutagene DNA

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<222> (1)..(35)
<223> Technical DNA/residues of the polylinker sequence

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<222> (36)..(522)
<223> Part of the 5' region (pck1) of the pckA gene

55 <220>
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<222> (523)..(542)
<223> Technical DNA/residues of the polylinker sequence

60 <220>
<221> misc_feature

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<222> (543)..(1105)
<223> Part of the 3' region (pck2) of the pckA gene
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<221> misc_feature
<222> (1106)..(1156)
<223> Technical DNA/residues of the polylinker sequence
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cacaaaaaga	taagtatatc	gtccgtgacg	ataccactcg	cgatactttc	tggtgggcag	180
acaaaggcaa	aggtaagaac	gacaacaaac	ctctctctcc	ggaaacctgg	cagcatctga	240
aaggctctgt	gaccaggcag	ctttccggca	aacgtctgtt	cgttgtcgac	gctttctgtg	300
gtgcgaaccc	ggatactcgt	ctttccgtcc	gtttctcac	cgaagtggcc	ttgcaggcgc	360
attttgtcaa	aaacatgttt	attcgccoga	gcgatgaaga	actggcaggt	ttcaaaccag	420
actttatcgt	tatgaacggc	gcgaagtgca	ctaaccgcga	gtggaaagaa	caggggtctca	480
actccgaaaa	cttcgtggcg	tttaacctga	ccgagcgcgt	gcaagccgaa	ttctgcagat	540
cctgaagatg	gcactatcga	ctttgatgat	ggttcaaaaa	ccgagaacac	ccgcgtttct	600
tatccgatct	atcacatcga	taacattggt	aagccggttt	ccaaagcggg	ccacgcgact	660
aaggttatct	tccgtactgc	tgatgctttc	ggcgtgttgc	cgccggtttc	tcgcctgact	720
gccgatcaaa	cccagtatca	cttcctctct	ggcttcaccg	ccaaactggc	cggtactgag	780
cgtggcatca	ccgaaccgac	gccaaccttc	tccgcttgct	tcggcgcggc	attcctgtcg	840
ctgcaccgga	ctcagtagcg	agaagtgtct	gtgaaacgta	tgcaaggcgc	gggcgcgcag	900
gcttatctgg	ttaacactgg	ctggaacggc	actggcaaac	gtatctcgat	taaagatacc	960
cgcgcattat	tcgacgccat	cctcaacggt	tcgctggata	atgcagaaac	cttcactctg	1020
ccgatgttta	acctggcgat	cccaaccgaa	ctgcggggcg	tagacacgaa	gattctcgat	1080
ccgcgtaaca	cctacgcttc	tccggaagcc	gaattctgca	gatatccatc	acactggcgg	1140
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<210> 4
<211> 1294
<212> DNA
<213> Escherichia coli
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<222> (1)..(3)
<223> Start codon of the delta pckA allele
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<220>
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<222> (1)..(598)
<223> 5' region of the delta pckA allele
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<220>  
<221> misc_feature  
<222> (599)..(618)  
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<220>
<221> misc feature
<222> (619)..(1291)
<223> 3' region of the delta pckA allele
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<222> (1292)..(1294)
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 catgatatcg tttacaaccc aagctacgac ctgctgtatc aggaagagct cgatccgagc 120
 ctgacagggt atgagcgcgg ggtgttaact aatctgggtg ccgttgccgt cgataccggg 180
 5 atcttcaccg gtcgttcacc aaaagataag tatatcgctc gtgacgatac cactcgcgat 240
 actttctggt gggcagacaa aggcaaagggt aagaacgaca acaaacctct ctctccggaa 300
 acctggcagc atctgaaagg cctggtgacc aggcagcttt ccggcaaacg tctgttcgtt 360
 gtcgacgctt tctgtggtgc gaaccgggat actcgtcttt ccgtccgttt catcaccgaa 420
 gtggcctggc aggcgcattt tgtcaaaaac atgtttattc gcccagcgca tgaagaactg 480
 10 gcaggtttca aaccagactt tatcgttatg aacggcgcga agtgactacta cccgcagtgg 540
 aaagaacagg gtctcaactc cgaaaacttc gtggcggtta acctgaccga gcgcattgcaa 600
 gccgaattct gcagatcctg aagatggcac tatcgacttt gatgatggtt caaaaaccga 660
 gaacaccgcg gtttcttctc cgatctatca catcgataac attgttaagc cggtttccaa 720
 agcggggccac gcgactaagg ttatcttctc gactgctgat gctttcggcg tgttgccgcc 780
 15 ggtttctcgc ctgactgccc atcaaaccga gtatcacttc ctctctggtt tcaccgccaa 840
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 20 ctcgattaaa gataccgcgc ccattatcga cgccatcctc aacggttcgc tggataatgc 1080
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25 <210> 5
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 <212> DNA
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30 <220>
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 <222> (376)..(714)
 <223> ORF ytfP

35 <220>
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 <222> Complement((461)..(727))
 <223> ORF yjfa

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 tcagagcgac agtgccgcaa tgacctcgat gctgattggt ttggggggtg cgcaaagtgg 120
 ccagattgtg ggtaaaatcg gcgagacggt tggcgtaagc aatttagcgc tcgacaccca 180
 45 gggagtaggc gactcctccc aggtagtggg cagcggctat gtattgccag gtctgcaagt 240
 gaaatacggc gtgggtatat ttgactctat agcaacactc acgttacgtt atcgccctgat 300
 gcctaagcta tatctggaag ccgtgtctgg tgtagaccag gcaactggatt tgctctatca 360
 gttcgagttt tagcaatgcg aatatattgtc tacggcagtt tacgccacaa acaaggcaac 420
 agtcaactgga tgaccaatgc ccagttactg ggcgatttca gtatcgataa ctaccagttg 480
 50 tatagcctgg gccactatcc aggcgcagtt ccggggaacg gaacgggtaca cgggtgaagt 540
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 ccgctcgatg gattaaagct aattgaaagc ggcgactggg tagacaggga taagtaacca 720
 tatgcatacg ccaccttcgg gtggcggttg tttttgcgag acgactcgca ttctgttttg 780
 55 taattccctc accttttgc tttctctccg agccgctttc catatctatt aacgcataaa 840
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 60 gcttgatgaa ctgctggcac agtgtgacat gaacgccgcg gaacttagcg agcaggatgt 1140
 ctgggggtaaa tccaccctcg cgggtgacga aatatggtaa agaaaagtga atttgaacgg 1200
 ggagacattg tgctgggttg ctttgatcca gcaagcggcc atgaacag 1248

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<222> (1)..(630)
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 <220>
 5 <221> misc feature
 <222> (631)..(1158)
 <223> 3' flank of the ytfP-yjfa region

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 10 <221> misc feature
 <222> (376)..(378)
 <223> ATG codon of the truncated ORF ytfP

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 15 <221> misc feature
 <222> Complement((635)..(637))
 <223> ATG codon of the truncated ORF yjfa

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 20 ggcgatgtcg caacaagctg ccttgtctta tttgctacgt ggacaagggc tggagagcga 60
 tcagagcgac agtgccggcaa tgacctcgat gctgattggt ttggggggtg cgcaaagtgg 120
 ccagattgtg ggtaaaatcg gcgagacgtt tggcgtaagc aatttagcgc tcgacaccca 180
 gggagtaggc gactcctccc aggtagtggc cagcggctat gtattgccag gtctgcaagt 240
 gaaatacggc gtgggtatat ttgactctat agcaacactc acgttacgtt atcgccctgat 300
 25 gcctaagcta tatctggaag ccgtgtctgg tgtagaccag gcaactggatt tgctctatca 360
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 tacgcgcgcc agttgattca gacgccgtac tatgcatacg ccaccttcgg gtggcgttgt 660
 tttttgcgag acgactcgca ttctgttttg taattccctc accttttgct tttctctccg 720
 agccgctttc catatctatt aacgcataaa aaactctgct ggcattcaca aatgcgcagg 780
 ggtaaaacgt ttctgttagc accgtgagtt atactttgta taacttaagg aggtgcagat 840
 gcgtattacc ataaaaagat gggggaacag tgcaggtatg gtcattccca atatcgtaat 900
 35 gaaagaactt aacttacagc cggggcagag cgtggaggcg caagtgagca acaatcaact 960
 gattctgaca cccatctcca ggcgctactc gcttgatgaa ctgctggcac agtgtgacat 1020
 gaacgccgag gaacttagcg agcaggatgt ctgggggtaaa tccaccctcg cgggtgacga 1080
 aatatggtaa agaaaagtga atttgaacgg ggagacattg tgctgggttg ctttgatcca 1140
 40 gcaagcggcc atgaacag 1158

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 45 <213> Artificial sequence

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 pckA'5'-1
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 gatccgagcc tgacaggtta 20

 55 <210> 9
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 <212> DNA
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 60 <220>
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 pckA'5'-2

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	<210> 10	
	<211> 22	
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10	<213> Artificial sequence	
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	<210> 11	
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25	<223> Description of the artificial sequence: Primer	
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30	ccggagaagc gtaggtgtta	20
	<210> 12	
	<211> 20	
	<212> DNA	
35	<213> Artificial sequence	
	<220>	
	<223> Description of the artificial sequence: Primer Gdh1	
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45		
	<210> 13	
	<211> 20	
	<212> DNA	
	<213> Artificial sequence	
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50	<223> Description of the artificial sequence: Primer Gdh2	
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	<213> Artificial sequence	
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25 <210> 16
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30 <220>
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40 <210> 17
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45 <220>
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aataccagcc cttgttcgtg 20

55 <210> 18
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60 <220>
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65 <400> 18
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70 <210> 19
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75 <220>
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000425 BT AL1

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